II. Status of the Claims

Claims 1-35 were previously pending in the present application, with claims 1 and 24 being the independent claims. In the Office Action dated March 26, 2002, the Examiner had objected to claims 5-23 and 28-35 and had rejected claims 1-4 and 24-27.

Currently, claims 1-38 are pending in the application, with claims 1 and 24 being the independent claims.

The Examiner states that the papers submitted under 35 U.S.C. § 119 (a) – (d) have been placed on record in the file. Applicants thank the Examiner for placing the papers on record.

III. Objection to Claims 5-23 and 28-35 Under 37 C.F.R. § 1.75(c) Is Accommodated

The Examiner objects to claims 5-23 and 28-35 under 37 C.F.R. § 1.75(c) as being in improper form because a multiple dependent claim cannot depend from any other multiple dependent claim. The Examiner did not examine these claims further on the merits.

The present application is the United States national phase of PCT/GB99/038309, which claims priority to 9825215.8 filed in the United Kingdom. Applicants hereby amend the claims in accordance with United States patent practice, thereby rendering the Examiner's rejection moot, and respectfully reiterate the request for examination of claims 5-23 and 28-35 on the merits.

IV. Rejection of claims 1-4 and 24 Under 35 U.S.C. § 102(b) Is Traversed

In the Office Action at pages 2-3 (paragraph 4), the Examiner has rejected claims 1-4 and 24 as being anticipated by JP 03101688. This rejection is respectfully traversed. The Examiner stated that the abstract of JP 03101688 taught an

aqueous mixture comprising a chaotrope (guanidinium salt) and butanol in a method of isolating plasmid DNA from an aqueous mixture of plasmid DNA and genomic DNA by adding a chaotrope (guanidinium salt) and butanol, then isolating the plasmid. The aqueous solution was at a basic pH. [Office Action, paragraph 4].

Applicants respectfully submit, however, that neither the abstract, nor the remainder of JP 03101688 anticipated claims 1-4 or claim 24.

Claim 1 currently reads as follows:

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:
   (i) extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA; and
   (ii) recovering the plasmid DNA from the butanol.

The Examiner has cited the abstract of JP 03101688. The abstract describes a method by which nucleic acids can be separated from samples or fermentation cultures by treating the sample with a protein denaturant (e.g., guanidine salt, urea, etc.) and then with a substance selected from ethanol, propanol, butanol, pentanol, and hexanol for precipitation or removal. An example is given in which a plasmid-transformed *Escherichia coli* culture medium was treated with guanidine-HCl and stirred with isopropanol. The sample was centrifuged, and the precipitate was washed twice with ethanol, dissolved in Tris buffer (pH 8.0) containing 1 mM EDTA and centrifuged again. The resulting supernatant was treated with ethanol and centrifuged to yield DNA.

The abstract of JP 03101688 describes a method for the separation of **nucleic acids** from **samples or cultures - not** the separation of **plasmid DNA** from a **DNA containing material** which comprises plasmid DNA and **genomic DNA**, as outlined in claim 1.

Claim 1 of the present application requires the extraction of plasmid DNA into butanol, which is water-immiscible. The specification of the present application states:

> In a preferred arrangement, the organic solvent is capable of **selectively** supporting the **plasmid** DNA with the **exclusion** of **genomic** DNA present in the plasmid DNA containing material. [p. 2, ll. 22-25, emphasis added.]

It also notes:

> In extraction step (i), the DNA containing material is mixed with the reagents under conditions to denature the genomic DNA typically whereby the **plasmid** DNA is partitioned into an **organic phase** and the **genomic** DNA is partitioned into an **aqueous phase**. [p. 2, l. 33 - p. 3, l. 1, emphasis added.]

In addition, it states:

> [I]t is thought that differential solubility between plasmid and genomic DNA under denaturing condition may result in plasmid DNA in an undenatured or reversibly denatured state partitioning into the organic phase. In contrast denatured genomic DNA partitions into the aqueous phase. [p. 3, ll. 18-23.]

However, the example in the abstract of JP03101688 describes isolation of a plasmid from an *E. coli* culture medium by treatment with guanidine-HCl stirred with isopropanol (not butanol) and centrifuged to form a **precipitate**. There is **no** discussion of the **separation** of **plasmid** DNA from **genomic** DNA. Likewise, there is no discussion in the specification of JP 03101688, a translation of which is provided, of the separation of different types of DNA. (Applicants respectfully note that the page numbers used in this Amendment discussion refer to the page numbers of the translation of the specification of JP 03101688.) Of the five Practical Examples, only Practical Example 4 (pp. 9-10) uses secondary butyl alcohol, and this example focuses on isolation of DNA from K562 human leukocyte cancer cells, which are not described as containing a plasmid, because they are **not plasmid-containing** cells. The remaining examples, including the *E. coli* example (Practical Example 3) mentioned in

the abstract, use either isopropanol (Practical Example 3) or ethanol (Practical Examples 1, 2, and 5).

In addition, Practical Example 4 does not disclose step ii of claim 1 with regard to recovery of the plasmid DNA from the butanol phase. In Practical Example 4, the butanol was used to precipitate DNA. **DNA** was **recovered from the precipitate**, rather than from the solvent, and, again, there is no mention of any plasmid DNA in this Example, because this Example does not use plasmid-containing cells.

According to the methods of the present invention, the **butanol and aqueous phases are immiscible** (see page 3, ll. 18-30), and the **plasmid DNA and genomic DNA partition differently** into these two immiscible phases, such that plasmid DNA is soluble in the organic solvent, but genomic DNA is not. While the present application also provides methods (e.g., claim 15) for precipitating plasmid DNA from the butanol, this precipitation occurs only after the plasmid DNA has been solubilized in the butanol phase and requires addition of a precipitating agent.

JP 03101688 mentions the use of butanol to precipitate DNA, but **does not teach** either the separation of plasmid DNA from genomic DNA or the solubility of plasmid DNA in butanol. It **does not even remotely suggest** the desirability of either the separation of types of DNA or the solubility of plasmid DNA in butanol, let alone describe a means of achieving either feature.

Claim 1 requires the extraction to take place "under conditions to denature the genomic DNA." While the abstract describes use of a **protein** denaturant, it does **not** address the issue of **genomic DNA** denaturation, much less discuss its significance. A protein denaturant would not necessarily suffice as a denaturant for genomic DNA.

Likewise, the JP 03101688 specification does not discuss denaturation of genomic DNA. Instead the specification focuses on the concentration limits required to enable the nucleic acid to form a precipitate upon the addition of the alcohol:

> The protein denaturing agent is added so as to give a concentration capable of denaturing the protein in the sample at the time when it is added to the sample, and such that thereafter the nucleic acid forms a precipitate on addition of the alcohol. [p. 5].

Thereafter the specification of JP 03101688 describes parameters for various protein denaturing agents (pp. 5-6). The specification also states:

> By means of the above operations, the **nucleic acid** contained in the sample forms a **precipitate**, whereas the **protein** is present **in the solution** in the dissolved state owing to the action of the protein denaturing agent. Consequently, after the method of the present invention has been implemented it is possible to extract or remove the nucleic acid for example by performing a centrifugal separation or membrane separation. [pp. 6-7, emphasis added].

Clearly, JP 03101688 is directed toward the isolation of **precipitated nucleic acids** from **"dissolved" proteins** and, **in contrast to** the present invention, is **not** concerned with the separation of **one type of nucleic acid in one phase** from **another type of nucleic acid in a different phase**.

The example in the JP03101688 abstract never states whether the plasmid DNA from the *E. coli* culture medium is isolated from the genomic *E. coli* DNA and how this isolation is achieved, nor does the abstract raise the issue in any way. In fact, the abstract describes the separation of "nucleic acids...from samples" and **does not even suggest** that the **separation of different types of DNA** from each other would be **desirable**. Moreover, the specification of JP03101688 likewise never addresses the separation of different types of DNA. Practical Example 3 (p. 9), which describes the above isolation of DNA from the plasmid-transformed *E. coli* strain, merely focuses on whether the resulting DNA can be subjected to various

restriction enzyme and polymerase reactions without inhibition and contains no discussion of genomic DNA. Practical Examples 1, 2, and 4 deal with human cancer cells and make no mention of plasmids. Practical Example 5 uses M13 phage. Thus, JP03101688 did not anticipate, or suggest, the invention of claim 1.

Claim 2 (amended) claims the "method of claim 1, wherein the conditions to denature the genomic DNA comprise basic conditions or a temperature of at least 65°C," and claim 3 (amended) is further limited to "basic conditions in which a base is present." The JP 03101688 abstract never addresses the issue of temperature for denaturation. As noted *supra*, the abstract also **never addresses** the issue of denaturation of **genomic DNA - only** the denaturation of **proteins**.

Claim 4 (amended) is dependent on claim 1, "wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol." The arguments for claim 1 likewise apply to claim 4.

Claim 24 (amended) reads:

> 24 (amended). An extraction mixture for selectively extracting plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water.

Again, the JP 03101688 abstract describes the separation of **nucleic acids** from **samples or cultures - not** an extraction mixture for **selectively** extracting **plasmid DNA** from a **DNA containing material** which comprises plasmid DNA and **genomic DNA**, as provided in claim 24. The abstract **never even suggests** that isolation of one type of DNA from another would be desirable. The specification likewise neither teaches nor suggests these features, as discussed *supra*.

In view of the foregoing remarks, Applicants respectfully assert that the present claims were not anticipated, or would not have been suggested, by JP 03101688. Therefore,

Applicants respectfully request reconsideration and withdrawal of the rejections made under 35 U.S.C. 102(b).

IV. Rejection of claims 1-4 and 24-27 Under 35 U.S.C. § 102(b) Is Traversed

In the Office Action at page 3 (paragraph 5), the Examiner has rejected claims 1-4 and 24-27 as being anticipated by, or in the alternative, obvious over, JP 03101688. This rejection is respectfully traversed. The Examiner states:

> JP 03101688 taught (see the abstract) a method of isolating plasmid DNA from an aqueous mixture of plasmid DNA and genomic DNA by adding a chaotrope (guanidinium salt) and butanol, then isolating the plasmid. The aqueous solution was at a basic pH.
>
> JP 03101688 did not explicitly teach that the basic pH of the solution was produced by adding sodium hydroxide. However, sodium hydroxide is a notorious and well known basic reagent used to produce a basic pH in a solution containing plasmid DNA and genomic DNA as shown in WO 99/61603 (see especially page 12). [Office Action, paragraph 5].

Claims 1-4 and 24 have been described, *supra*. Claim 25 claims the "extraction mixture of claim 24, which further comprises a base." Claim 26 claims the extraction mixture of claim 25, "wherein the base comprises a hydroxide." Claim 27 claims the mixture of claim 26, "wherein the hydroxide comprises sodium hydroxide."

WO 99/61603 describes a buffer, a kit and methods for "separating and/or isolating circular nucleic acids from a mixture having different species of nucleic acids other than circular nucleic acids" under alkaline conditions (pH > 8) with a solid matrix of silica material and "at least one chaotropic substance" (claim 1 and throughout). The specification describes the making of mixtures of linear and circular nucleic acids, which are then bound to silica material and the selective washing away of linear DNA with a chaotropic buffer at alkaline

pH (Examples 1-3; pp. 21-23). It also describes the use of multi-step protocols with multiple buffers used to isolate the genomic and plasmid DNA from transformed *E. coli* onto silica membrane, resin or particles (Examples 4-6; pp. 16-21 and 24) for the subsequent removal of the genomic DNA and isolation of the plasmid DNA. The specification describes the observation of Birnboim & Doly (Nucl. Acids Res. 7(6): 1513-1523 (1979)) concerning the finding that at pH 12.0-12.5 plasmid DNA remains undenatured, whereas chromosomal DNA is denatured. The specification then discusses the combined effects of a chaotrope in shifting this pH effect to lower and broader pH values.

In addition, the specification states:

> Furthermore, the invention takes advantage of the effect that under the conditions - presence of high molar chaotropic substances at alkaline pH - circular closed double stranded DNA (e.g., plasmid) but not linear DNA fragment (e.g., sheared chromosomal DNA) specifically binds to silica material. The precise pH may be adjusted with all kinds of pH buffers effective in this range of pH. Examples are phosphate buffers, glycine buffers, and boric acid/sodium hydroxide buffer. Due to its high buffer capacity in particular glycine buffers seem to be suitable for this purpose. [pp. 11-12].

While WO 99/61603 notes the desirability of isolating plasmid DNA from linear DNA and mentions a boric acid/sodium hydroxide buffer, however, it in no way suggests the separation of genomic and plasmid DNA in **separate phases** simply by using basic conditions to denature genomic DNA. Instead it requires **lengthy isolation** protocols using a number of buffers, followed by **binding to a silica matrix** as a condition for separation.

The present invention provides a simplified method (see, e.g., p. 2, ll. 1-3) for isolating plasmid DNA. As noted in the present application, many previously proposed methods require a series of steps or special apparatus (see generally p. 1), whereas "the present invention provides a 'one step' method which is simple to perform and which requires no specialised laboratory apparatus" (p. 2, ll. 17-19). The present invention requires no silica

matrix or lengthy isolation steps prior to the mixing of the DNA containing material with butanol, a chaotrope and water as described. For instance, Examples 1 and 2 of the present application simply describe the pelleting of cells by centrifugation followed by resuspension in TE (pp. 5-8) prior to mixing with butanol, a chaotrope and water.

Applicants respectfully submit that neither the abstract, nor the remainder of JP 03101688, anticipated, or suggested, the invention as claimed in claims 1-4 or claims 24-27. Applicants have already discussed JP 03101688 at length, and the discussion, *supra*, also applies to this rejection. In addition, the use of basic conditions in WO 99/61603, even as background information, does not cure the deficiencies of JP 03101688, which neither teaches nor suggests separation of plasmid DNA from genomic DNA, let alone by selective denaturation of the genomic DNA only.

Moreover, the Examiner rejected claims 1-4 and 24-27 under 35 U.S.C. §102(b), which requires that all the elements of an anticipated claim be found in a **single reference**. JP 03101688 lacks each and every element of the claim, and its deficiencies cannot be supplied by the teachings of WO 99/61603, which requires significant additional steps for isolation, including the use of a silica matrix. There is no suggestion to combine the teachings of JP 03101688 (precipitation of DNA) with those of WO 99/61603 (use of a silica matrix to separate genomic DNA from plasmid DNA) to achieve the methods of the present invention (isolation of plasmid DNA from genomic DNA and other material by solubilization of plasmid DNA in butanol). Moreover, even if those references had been combined, this combination would not meet, describe, or suggest the invention of the present claims.

In view of the foregoing remarks, Applicants respectfully assert that the present claims were not anticipated, and would not have been suggested, by JP03101688, either with or without WO 99/61603. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejections made under 35 U.S.C. 102(b).

VI. Conclusion

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

Although it is not believed that any additional fee is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

Date: August 26, 2002

Kathryn A. Piffat, Ph.D.

David G. Conlin, (Reg. No. 27,026)
Kathryn A. Piffat, Ph.D., (Reg. No. 34,901)
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group
EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, Massachusetts 02209
Telephone: 617-439-4444
Facsimile: 617-439-4170

APPENDIX I

REVISIONS OF CLAIMS PURSUANT TO REVISED RULE § 1.121

Pursuant to Revised Rule § 1.121(c)(1)(ii), the revisions of claims 2-7, 9-10, 13, 15, 19, 21, 23-24, 28-29, 32, and 34 are detailed as follows (marked new claims not required):

2 (amended). The method of claim 1, wherein the conditions to denature the genomic DNA comprise basic conditions or a temperature of at least 65°C.

3 (amended). The method of claim 2, wherein the conditions to denature the genomic DNA comprise basic conditions in which a base is present.

4 (amended). The method of claim 1 [any preceding claim], wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.

5 (amended). The method of claim 1 [any preceding claim], wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and mixtures thereof.

6 (amended). The method of claim 1 [6], wherein the chaotrope comprises guanidine hydrochloride.

7 (amended). The method of claim 3 [any one of claims 3 to 6], wherein the base comprises a hydroxide.

9 (amended). The method of claim 3 [any one of claims 3 to 8], wherein the butanol, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA containing material.

10 (amended). The method of claim 1 [any preceding claim], wherein the amount of butanol is in the range from 20 to 70% based on the volume of the combination of butanol, chaotrope and water.

13 (amended). The method of claim 1 [any preceding claim], wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of butanol, chaotrope and water.

15 (amended). The method of claim 1 [any preceding claim], wherein the recovery step (ii) comprises mixing the DNA containing butanol phase with a precipitating agent that can precipitate the plasmid DNA from the butanol, and separating the precipitated plasmid DNA from the butanol.

19 (amended). The method of claim 15 [any one of claims 15 to 18], wherein the precipitating agent further comprises an acetate salt.

21 (amended). The method of claim 1 [any preceding claim], which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.

23 (amended). The method of claim 1 [any preceding claim], wherein the DNA containing material comprises a lysed or unlysed bacterial culture.

24 (amended). An extraction mixture for selectively extracting plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water.

28 (amended). The extraction mixture of claim 24 [any one of claims 24 to 27], wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.

29 (amended). The extraction mixture of claim 24 [any one of claims 24 to 28], wherein the butanol constitutes from 20 to 70% based on the volume of the extraction mixture.

32 (amended). The extraction mixture of claim 24 [any one of claims 24 to 31], wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and mixtures thereof.

34 (amended). The extraction mixture of claim 24 [any one of claims 24 to 33], wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.